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ONR FINAL TECHNICAL REPORT

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PRINCIPAL INVESTIGATOR: Kenneth J. Rothschild
INSTITUTION: Boston University
GRANT TITLE: *Biophysical Study of Archaebacteria Biomembrane and Possible Application in Biomaterials*
REPORTING PERIOD: July 1, 1988-Dec. 31, 1991

I. OVERALL PROJECT OBJECTIVES

To investigate the molecular mechanisms and basis for structural stability of membrane proteins in Archaebacteria. An additional objective is to study the utilization of these membranes in the production of new materials which are fabricated on the nanometer scale.

II. SUMMARY OF ACCOMPLISHMENTS OF PROJECT:

Our research accomplishments are summarized below in three major areas:

1. Structure-Function Studies on Archaebacteria membrane proteins: Our studies in collaboration with the Khorana laboratory at MIT on the biophysical properties of site-directed mutants of **bacteriorhodopsin** have led to an increasingly detailed model of the structure and proton transport mechanism of this membrane protein (1-6,7-12,14-20,23-25; Technical Reports #1-3). Additional progress has been made in studying two other related halobacterial membranes proteins, **halorhodopsin** (17) , which functions as a chloride pump, and **sensory rhodopsin I** (24) , which serves as a photoreceptor for phototaxis in bacteria. In general, this work constitutes one of the most detailed investigations of the functional mechanism of membrane proteins to date, and provides critical information for future attempts at using these biomolecular materials.

Technical Reports 1-3 and the above mentioned publications detail the application of FTIR, resonance Raman and UV/Vis spectroscopy and information gained about the bR proton pump mechanism and the role of the key amino acid residues. An important aspect of this work has been the identification of an active site in light adapted bR that includes an electrostatically neutral arrangement of the protonated Schiff base, Asp-85, Asp-212 and Arg-82 in agreement with an electron diffraction derived model (3,9,20,27). The disruption of this active site upon formation of the M intermediate due to protonation of Asp-85 from the Schiff base is believed to cause ejection of a proton into the outer medium due to the electrostatic unshielding of the positively charged residue Arg-82. FTIR difference spectra of the late photocycle of bR have also been obtained

during the project period (15,20,25) which shows that Asp-96 deprotonates while during N decay Asp-96 reprotonates. Evidence was also found for a proton wire which is active in movement of a proton during the M to N transition of the photocycle from Asp-96 to the Schiff base (20).

2. Development of new biophysical techniques to study Archaebacteria membrane proteins: An important aspect of the supported research has been the development of new techniques which can be used to study the structure and function of membrane proteins at the level of individual amino acids . Most important in this area is the introduction of **time-resolved FTIR** to study conformational changes that occur in response to a stimulus (i.e. a flash of light). In publications (14,15 and 25), we describe the use of stroboscopic FTIR to measure changes in the photocycle of bacteriorhodopsin. A combination of **polarized FTIR and hydrogen/deuterium exchange** has also been utilized for the first time to study the structure of membrane proteins (10). This work should provide a basis for future studies of Archaebacteria protein structure and stability. In a related work (21-22), it was demonstrated how ATR can be used to investigate the conformational changes of a membrane protein (acetylcholine receptor) to a chemical stimulus (acethylcholine) when the stimulus is introduced into a flowing solution.

3. Biomolecular Material Development: Structure-function studies on mutants of bacteriorhodopsin (see 1.) has provided information which can be used to alter this molecules properties for the development of **optoelectronic materials.** Mutants have been identified which have blocks at specific steps in the photocycle (19, 20 and 25) can be useful in holographic recording media and optical memories. Increasing knowledge of how retinal isomerization couples to proton movement should lead to further improvements in this area. Progress has also been made in using two-dimensionally crystalline membrane protein arrays as templates for **nanometer patterning.** Scanning tunneling microscopy (STM) measurements have been made on the S-layer from the *Sulfolobus Acidocaldarius* in collaboration with the N. Clark and K. Douglas at the University of Colorado (7,13). These studies have led to information about the ultrastructure of S-layer and thin metal films patterned on the nanometer level with these membranes.

III. PUBLICATIONS AND REPORTS:

Patents:

1. U.S. Patent # 4,802,951 . Clark, N.A., Douglas, K., and Rothschild, K.J. "Method for Parallel Fabrication of Nanometer Scale Multi-Device Structures"

Publications

1. K.J. Rothschild, M.S. Braiman, O. Bousche, Y-W. He, and W.J. De Grip "FTIR spectroscopy: the detection of individual chemical groups in complex biomolecules" *Proc. SPIE 1057*, 44-48 (1989).

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Acid 212 with Tyrosine 185 and Possible Role in The Proton Pump Mechanism" *J. Biol. Chem. 265*, 16985-16991 (1990)

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11. M. Dunach, T. Marti, H. G. Khorana and K. J. Rothschild, ``Bacteriorhodopsin Mutants of Arg-82, Asp-85, Tyr-185 and Asp- 212 Do Not Undergo Normal Light-Dark Adaptation" *Proc. Natl. Acad. Sci. USA 87*, 9873-9877 (1990).

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13. K. Douglas, N. A. Clark, K.J. Rothschild ``Biomolecular/solid-state nanoheterostructures" *Appl. Phys. Lett. 56*, 692-694 (1990)

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18. Swaerjo, M. Rothschild, K. J. Nappi, B., Lane, A. and Gold, H. "Infrared Fiber Optic Sensors: New Applications in Biology and Medicine", *SPIE, 1437* 60-65 (1991)

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Technical Reports:

1. Annual Progress Report #1-July 1, 1988-June 31, 1989

2. Annual Progress Report #2-July 1, 1989-June 31, 1990

3. Annual Progress Report #3-July 1, 1990-Dec. 31, 1991